

# Antipredator behavior and cue recognition by multiple Everglades prey to a novel cichlid predator

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## Summary

Novel predator introductions are thought to have a high impact on native prey, especially in freshwater systems. Prey may fail to recognize predators as a threat, or show inappropriate or ineffective responses. The ability of prey to recognize and respond appropriately to novel predators may depend on the prey's use of general or specific cues to detect predation threats. We used laboratory experiments to examine the ability of three native Everglades prey species (Eastern mosquitofish, flagfish and riverine grass shrimp) to respond to the presence, as well as to the chemical and visual cues of a native predator (warmouth) and a recently-introduced non-native predator (African jewelfish). We used prey from populations that had not previously encountered jewelfish. Despite this novelty, the native warmouth and non-native jewelfish had overall similar predatory effects, except on mosquitofish, which suffered higher warmouth predation. When predators were present, the three prey taxa showed consistent and strong responses to the non-native jewelfish, which were similar in magnitude to the responses exhibited to the native warmouth. When cues were presented, fish prey responded largely to chemical cues, while shrimp showed no response to either chemical or visual cues. Overall, responses by mosquitofish and flagfish to chemical cues indicated low differentiation among cue types, with similar responses to general and specific cues. The fact that antipredator behaviours were similar toward native and non-native predators suggests that the susceptibility to a novel fish predator may be similar to that of native fishes, and prey may overcome predator novelty, at least when predators are confamilial to other common and longer-established non-native threats.

*Keywords:* predation, antipredator behaviour, non-native, cues, fish.

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## 1. Introduction

The susceptibility of prey to predation risk is strongly influenced by the prey's ability to detect and respond to predation threats (Hoare et al., 2007; Ramo-Jiliberto et al., 2007; Smith et al., 2008a). If the predation threat is novel, the ability of prey to both recognize and respond to predators may be limited (Gamradt & Kats, 1996). For instance, a lack of evolutionary history between a non-native predator and native prey may cause prey to be naïve to a non-native predator's threat (i.e., naïve prey hypothesis; Smith et al., 2008b; Sih et al., 2010). Even if non-native predators are similar to native predators (e.g., both are fish), differences in predator archetypes due to variation in morphological and behavioural foraging adaptations can result in prey naiveté (Cox & Lima, 2006). This naiveté can contribute to the high consumptive effects of non-native predators introduced to isolated ecosystems such as islands and freshwater systems (Vermeij, 1991; Cox & Lima, 2006; Nannini & Belk, 2006; Wohlfahrt et al., 2006; Salo et al., 2007; Sih et al., 2010). Thus, in order to better understand the overall effects of non-native predators, we must gain a mechanistic understanding of how prey recognize and respond to new threats, and may overcome predator novelty.

Prey naiveté toward non-native predators may arise from three sequential mechanisms: (i) the failure of prey to detect or recognize novel predators as a threat, (ii) their inability to respond appropriately and/or (iii) their inability to effectively evade novel predators despite their appropriate response (Banks & Dickman, 2007). For instance, the lack of experience with predators altogether among island-endemic species often means that prey lack behavioural responses to introduced predators (Wiles et al., 2002; Blackburn et al., 2004). In other cases, prey recognized the predator as a threat, but show the wrong responses (e.g., crypsis against scent-hunting cursorial predators; Banks & Dickman, 2007). Thirdly, prey may recognize and respond with appropriate behaviours, but these may not be as effective against novel predators (Hudgens & Garcelon, 2011). For example, prey may increase use of higher cover habitats, but predation may still be high (Kinnear et al., 2002). Cox & Lima (2006) suggest that a lack of novel predator recognition may be the most damaging form of prey naiveté. A prey's failure to recognize a novel predator may inhibit its antipredator responses, or weaken such defences if recognition is delayed (Cox & Lima, 2006, but see Rehage et al., 2009).

Predator recognition hinges on the sensory information used to assess risk, which is often visual, chemical or a combination of the two (Hartman &

Abrahams, 2000; Mathis & Vincent, 2000; Chivers et al., 2001; Wisenden et al., 2004; Smith et al., 2008b). Cues used in predator detection may also vary from general to specific (Brown, 2003; Webb et al., 2009). Specific cues can effectively label a predation threat by revealing the predator's identity (i.e., a predator's particular odour or specific shape, Magurran & Girling, 1986; Kats & Dill, 1998; Wisenden & Chivers, 2006), while general cues are produced by a relatively broad range of information, and are not linked to a specific predator (i.e., damage or diet cues, habitat cues, broad visual cue — large moving object, Dill, 1974; Sih, 1986; Garcia et al., 1992; Gelowitz et al., 1993; Orrock et al., 2004). Specific cues allow prey to moderate antipredator responses by minimizing the use of costly antipredator behaviours against low-risk predators (Ramos-Jiliberto et al., 2007). At the same time, prey that rely on specific cues may be at a disadvantage when faced with novel, non-native predators not previously encountered (Sih et al., 2010). Here, their ability to overcome predator novelty will be strongly dependent on cue association, and rapid learning (e.g., Ferrari et al., 2007).

In our study, we compared the mechanisms of cue utilization, predator recognition, and antipredator response among native taxa faced with either a sympatric native predator or an allopatric non-native predation threat. Our intent was to gain a better understanding of the risk posed by novel, non-native predators, and of the variation in the susceptibility of native prey to newly-arrived predators. In three laboratory experiments, we compared predation rates, antipredator behaviours, and cue use by three native Everglades taxa in response to the threat of non-native African jewelfish, *Hemichromis letourneuxi*, and that of a common native centrarchid predator, the warmouth, *Lepomis gulosus*. The small-body size, piscivorous diet, and aggressive behaviour of the jewelfish make it a likely competitor to native centrarchids, which are the dominant mesoconsumers in the system (Loftus & Kushlan, 1987; Heymans et al., 2002; Rehage & Trexler, 2006; Schofield et al., 2007). With Everglades National Park (ENP) currently home to 17 non-native fishes species, many of them predators (Loftus et al., 2000; Trexler et al., 2000; Shafland et al., 2008), there is a need to better understand interactions among native and non-native taxa. To date, few studies have documented any significant ecological effects from fish introductions in ENP, which has led to conflicting perspectives on the overall impact of non-native aquatic taxa across the system (Shafland, 1996; Trexler et al., 2000).

We focused on the African jewelfish because, due to the recentness of the invasion in ENP (since 2000, J. Kline, pers. commun.; Courtenay et al., 1974; Shafland et al., 2008), we are able to track its spread; and its current patchy distribution creates heterogeneity in prey naiveté throughout the landscape. Thus, we are able to examine interactions among jewelfish and native Everglades prey that have not previously encountered them in nature and are, therefore, 'naïve' to their threat. Further, the majority of the non-native taxa in the Everglades are cichlids and, thus, there is an interest in learning how novel of a threat newly-arrived cichlids predators are. Ferrari et al. (2007) showed that prey may be able to generalize their antipredator response to closely-related predators in the absence of experience. At the same time, variation in predator hunting behaviour and habitat domain even among closely-related predators can create some level of predator novelty (Rehage et al., 2009). Here, we focused on three common native prey species: Eastern mosquitofish, *Gambusia holbrooki*, flagfish, *Jordanella floridae*, and riverine grass shrimp, *Palaemonetes paludosus*. These three species are widely-distributed in the Everglades, co-occur, and are among the most abundant prey of freshwater marshes (Turner et al., 1999; Trexler et al., 2001; Rehage & Trexler, 2006). They are also readily consumed by both non-native jewelfish (Rehage et al., 2009; Whitaker, unpubl. data) and native warmouth (W.F. Loftus, unpubl. data), but little is known about prey-specific vulnerability to piscine predators.

In the three experiments, we addressed four key questions: (1) Is the predation threat posed by non-native jewelfish similar to that posed by the native warmouth? (2) How do non-native predators and native predators interact to affect prey mortality? (3) Do prey exhibit the same antipredator responses to native and non-native predators? (4) What predator cues are prey using to detect these predators? In the first experiment, we examined the antipredator behaviour of each prey species to the presence of predators, as well as predator behaviour, and predation rates. We expected weaker antipredator responses by all three taxa to the novel jewelfish predator and, thus, higher predation rates by the non-native predator. We also expected to see variation in the vulnerability of the prey taxa to both predators, which we hypothesized would relate to their antipredator behaviour, habitat domain overlap with predators (Schmitz, 2007) and, thus, encounter rates. For instance, since both predators tend to be found low in the water column, we expected benthic

prey (shrimp and flagfish) to experience higher predation by both the predator types (Rehage et al., 2009; Whitaker, unpubl. data). In experiments 2 and 3, we assessed the prey's use of chemical and visual cues, both general and specific. We expected that the antipredator response of prey would relate to the use of general or specific predation cues in predator detection. We expected native prey to respond to the cues of the native predator more strongly than those of the non-native predator. Further, we hypothesize that if prey were unable to smell or recognize African jewelfish visually as a predator, they could still respond appropriately if they relied on general cues for predator detection (i.e., conspecifics damage cues). From these experiments, we hoped to gain new insights into the mechanisms underlying variation in the vulnerability of Everglades aquatic taxa to recent invasions.

## **2. Methods**

### *2.1. Study organisms*

For all experiments, native and non-native predators were collected from freshwater marshes in ENP and southern Big Cypress National Preserve where jewelfish and native centrarchids co-occur. The three prey species were collected exclusively in northern Water Conservation Area 3A (WCA3A), where jewelfish have not yet invaded. Additional warmouth were also collected at this site. We collected predators and prey using unbaited minnow traps deployed overnight (2.5-cm openings, 3-mm mesh), in addition to D-frame dip nets used for collecting prey (1-mm mesh). Prior to experiments, predators were kept separately for 3–45 days at approximately equal densities in 795-l outdoor tanks at Nova Southeastern University Oceanographic Center, Dania, FL, USA. During this holding period, predators were fed a combination of live prey (including experimental prey), and earthworms obtained commercially. Prey species were kept separately by species at similar densities in 795-l outdoor tanks for 1–45 days prior to trials, and fed commercial flakes ad libitum.

### *2.2. Experimental design*

In each of the three experiments conducted in the study, we used a  $3 \times 4$  factorial design (3 species  $\times$  4 experimental treatments) to compare prey antipredator responses to the presence, chemical and visual cues of native and

non-native predators. Experiment 1 compared predation rates, and predator and prey behaviour, while experiments 2 and 3 examined prey behaviour in response to chemical and visual cues respectively. In all three experiments, data was collected on each prey species separately, and on a randomly-assembled group of six similarly-size individuals from each species (Rehage et al., 2009). For each experiment, we randomly selected a new group of six prey, such that prey were only used once. Four key prey behaviours were repeatedly assessed in the three experiments separately for each prey species: activity, grouping, vertical distribution, and use of habitat structure. Previous research shows that these are behaviours typically affected by predation risk, including in our focal species (Sih, 1986; Main, 1987; Crowl & Covich, 1994; Skelly, 1994; Smith & Belk, 2001; Davis, 2003; Carson and Merchant, 2005; Rehage et al., 2009). For instance, Carson & Merchant (2005) showed that a close relative of our shrimp species decreased activity and increased grouping in response to predation risk.

All trials were conducted in 12 56.8-l aquaria ( $50 \times 24.5 \times 40$  cm height) at a water depth of 33 cm using dechlorinated tap water at approximately  $25.7^\circ\text{C}$ . Each tank was provided with structural complexity in the form of artificial vegetation, covering 1/3 of the tank area. The artificial vegetation consisted of black plastic strips ( $4 \times 22$  cm) attached to a weighted plastic grid ( $16 \times 16$  cm), which sat on the bottom and to one side of the tank. This amount of structure corresponds to a plant stem density of approximately  $484$  stems/ $\text{m}^2$ , which falls within the range found in Everglades marshes ( $18$ – $677$  stems/ $\text{m}^2$ ; Jordan et al., 1997). To minimize observer effects in the first experiment, tanks were covered on all four sides with a white vinyl covering, and observations were conducted through mirrors positioned above tanks. For the later cue experiments, tanks were covered on three sides only, and observations were conducted laterally from behind a blind.

At 12 h prior to the start of each experiment, all feeding in stock tanks was suspended in order to standardize hunger levels, and six prey of each species were randomly selected from stock tanks, and isolated into groups in 5.7-l containers (separately by species). At 15 min before trials, the prey group was randomly assigned to a treatment and replicate tank. Prey sizes, based on a random sample from the three experiments ( $N = 15$  for each spp) averaged ( $\pm$ SE)  $13.3 \pm 0.5$  mm standard length (SL) for mosquitofish,

19.1 ± 0.7 mm SL for flagfish and 8.7 ± 0.3 mm carapace length for grass shrimp.

Behavioural observations were conducted through a series of discrete spot-checks by a single observer positioned approximately one meter in front of each tank (Mathis & Smith, 1993b). This type of scan sampling, where study subjects are rapidly scanned at regular intervals, is appropriate when recording few and conspicuous behaviours such as in our study (e.g., activity; Martin & Bateson, 2007). For experiment 1, 10 scans were conducted in rounds, with the observer observing all tanks over a period of 15–20 min, then returning to the first tank for another round, and repeating this for 10 rounds (approximately 3 h of total observation). For the cue experiments, the 12 spot-check observations were done consecutively with the observer performing all observations at one tank and then moving to the next tank (6 were conducted pre- and 6 post-cue addition). Here, observations were conducted approximately every 2 min, except observations 6 and 7, which were conducted immediately pre- and post-cue addition (within 1 min). Total observation periods for experiments 2 and 3 were approximately 12 min. For all observations, we recorded four key prey behaviours of interest: activity, microhabitat use (use of the habitat structure and water column) and group size. At each spot check, we scored the activity and microhabitat use of each individual in the group, and then averaged the score for the group. Activity was scored as 0 if immobile, 1 = slow, 2 = medium and 3 = high. We considered high activity to be a darting or active escape response at high speed from a predator. Medium activity was a continuous uninterrupted swimming pattern (longer than 3 s), while slow swimming involved a cautious ‘stop and go’ swimming behaviour.

We assessed two components of microhabitat use: the prey’s vertical distribution in the water column, and the use of structure. To determine vertical distribution, we divided the water column into equal-sized horizontal layers (0 = bottom, 1 = middle, 2 = top), recorded the location of each fish at each spot check and averaged for the 6 fish in the group. Marks on each corner of tanks, which divided the 33 cm water column into three 11-cm zones, aided the observer in scoring use of the water column. To quantify habitat structure use, we counted the number of prey within the structure at each spot check. Lastly, for the schooling or grouping behaviour, we recorded the occurrence of a group at each observation (group present = 1, group absent = 0). Prey were considered to be in a social group if at least four of the six individuals

were closer than 2 body lengths (Rehage et al., 2009). All observations were conducted between 11 am and 2 pm.

### 2.3. *Experiment 1: predator-prey interactions*

Here, we crossed the three prey species with four treatments in a replacement series design (Sih et al., 1998): (NP) a no predator control, (WW) two warmouth, (JJ) two jewelfish, and (WJ) one warmouth + one jewelfish. Trials were conducted in two time blocks (March 31–April 4, 2008 and April 10–14, 2008). For both blocks, a single replicate was tested each day over the 5-day period (4 treatments  $\times$  3 prey spp  $\times$  5 replicates per block  $\times$  2 blocks = 120 experimental units). Each predator was used once during each block, returned to stock tanks, randomized, and then used again in the second block (9 predators  $\times$  2 predators spp  $\times$  5 replicates = 90 total predators). Prey species were tested only once (120 experimental units  $\times$  6 individuals/group = 720 total prey).

Previous studies have shown that prey are capable of responding to dietary cues released by predators that have consumed conspecifics (Mathis & Smith, 1993a; Chivers & Mirza, 2001). To eliminate the effects of these cues in the experiment, predators were maintained on a diet consisting solely of commercial earthworms for five days prior to the start of trials (Gelowitz et al., 1993; Mathis & Smith, 1993b). Previous studies have shown that digestions rates for piscivorous and crustacean-consuming predators are less than 48 h when water temperatures are approximately 22.7°C (Kitchell & Windell, 1968), which is close to the average temperature of 24.2°C in our study. Following this five-day diet flushing period, predators (warmouth  $65.6 \pm 1.7$  mm SL,  $N = 45$ , and jewelfish  $56.7 \pm 1.0$  mm SL,  $N = 45$ ) were randomly selected and isolated in 5.7-l containers the evening before trials. We were careful to conduct water changes during this period, and not transfer any of the water of the predator stock tank or isolation container to experimental tanks.

In addition to the prey's behaviour, we recorded predator activity and microhabitat use using the same scoring scheme used for the prey. At the beginning of trials, prey groups were released into aquaria first, allowed 15 min to acclimate, and then predators were added. Observations started 10 min after predator release. At the conclusion of all behavioural observations on trial days, we assessed overnight prey mortality. To prevent prey depletion



in tanks, an additional six prey individuals of the same species and size were added, for a total of 12 prey individuals per tank. Rehage et al. (2009) showed overnight predation rates of 7 mosquitofish using a similar setup. Prey were added following the observation period (3–4 pm), and mortality checks were done the following morning (7–8 am). If any of the original prey were consumed during the behavioural observations (only 40 of 720 prey were consumed over the observation period), prey were replaced before assessing overnight mortality, but not during the observation period (Rehage et al., 2009).

#### *2.4. Experiment 2: prey responses to chemical cues*

The three native Everglades prey species were tested in four chemical cue treatments: (NP) a no-cue control, (G) a general cue consisting of the odor of injured conspecifics, (W) specific chemical cues from the native war-mouth, and (J) specific chemical cues from the non-native jewelfish. Chemical cue trials were conducted over a 5-day period with 2 replicates per day (4 treatments  $\times$  3 prey species  $\times$  2 replicates per day  $\times$  5 days = 240 experimental units). Trials were conducted between August 23 and September 4, 2008. Each aquarium was provided with the same structural complexity described earlier, plus sodium zeolite chips at the bottom of the tank to remove ammonia, and aeration (vinyl tubing and an airstone) for cue release (Mathis & Smith, 1993b; Chivers & Smith, 1994). This airstone apparatus was positioned in the lower third of the water column at the opposite end of the tank from the habitat structure. We injected 60 ml of chemical cue into the vinyl tubing with a syringe for diffusion into the tank, and conducted observations 6 and 7 of the 12 observations within a minute pre- and post-cue addition (Mathis & Smith, 1993b; Brown & Smith, 1997; Chivers et al., 2001).

For the specific cues, six randomly-selected predators of each species were used to prepare predator odours. As in the first experiment, predators were maintained on a diet consisting solely of commercial earthworms for five days prior to the stimulus collection to remove dietary cues. On the fifth day of feeding, each predator was transferred to 5.7-l plastic containers containing new dechlorinated tap water. These chambers had a single air stone, but no filtration system. After 2.5 days, the predators were removed and water samples were collected from each predator chamber, and frozen into separate 120-ml units at  $-20^{\circ}\text{C}$  for later use (Gelowitz et al., 1993;

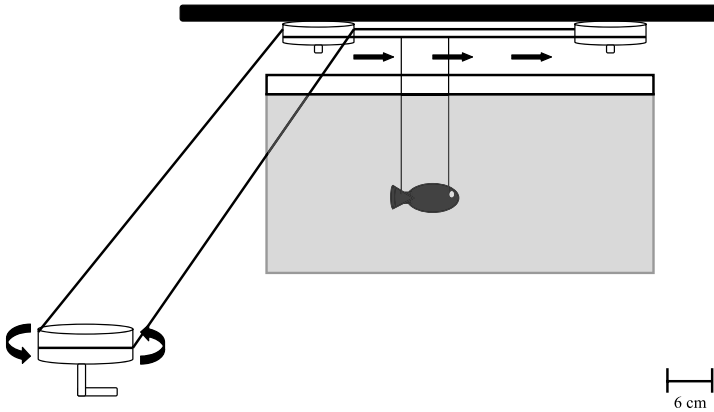
Brown & Godin, 1999; Kusch et al., 2004). Predator cues were not mixed and cue preparation was done twice over the five days of trials.

The general chemical cue was obtained from conspecific skin extracts. Thirty donors were randomly selected from each prey spp, and humanely sacrificed with a blow to the head. For the fishes, we removed the skin, and ground it up using a pestle and mortar to release the alarm signalling club cells (Pfeiffer, 1977; Wisenden, 2000). Because grass shrimp do not have these alarm cells, muscle tissue from beneath the carapace and tail was used instead (Magurran et al., 1996). Fish skin and shrimp tissue were diluted to 0.5 g/500 ml with distilled water, and the suspension was filtered and separated into 18 120 ml-units and frozen at  $-20^{\circ}\text{C}$  (Magurran et al., 1996). Stimulus cues were prepared every 2.5 days. For the control, 60 ml aliquots of distilled water were frozen, and injected in a similar manner as chemical cues.

### 2.5. *Experiment 3: prey responses to visual cues*

Similar to the chemical cue experiment, treatments for the last experiment included: (NP) a no cue control, (G) general visual cues from a predator model, (W) specific visual cues from the native warmouth, and (J) specific visual cues from the non-native jewelfish. Trials were conducted over two five-day time blocks (October 29–November 1, 2008 and November 10–14, 2008). For both blocks, a single replicate of each treatment by species combination was tested each day (4 treatments  $\times$  3 prey spp  $\times$  5 replicates per block  $\times$  2 blocks = 120 experimental units). Predators were used only once in each block, returned to stock tanks, randomized, and then used again in the second block (30 jewelfish and 30 warmouth total).

For each trial day, the three warmouth and three jewelfish were isolated for a 12-h period in the 5.7-l containers prior to the experiment. On the day of trials, the random prey group and predator were placed in adjacent glass tanks (broad side, covered by a removable barrier), and allowed to acclimate for 15 min. We conducted trials in two adjacent 56.8-l aquaria (one containing the six focal individuals of a prey species and one containing a single live predator or predator model). To ensure that predators were visible to prey, structure was not provided in predator tanks. For the control, the tank adjacent to the prey did not contain a visual stimulus, but we removed the barrier at the beginning of each trial as done in predator treatments (Brown et al., 1997; Chivers et al., 2001). Six scans were conducted pre and six post



**Figure 1.** Diagram of the model used for the visual cue experiment. The predator model consisted of a wooden dowel shaped like a fish (60 mm SL), suspended in the water column at a depth of approximately of 11 cm, and moved using a pulley system. The tank containing the model was positioned adjacent to the prey tank and separated with a removable barrier, similar to the other treatments.

removal of the barrier (observations 6 and 7 were conducted within a minute of barrier removal).

For the general predator cue, we used a predator model that consisted of a wooden dowel shaped in the form of a fish of similar size as the focal predators (60 mm SL, Figure 1). The use of models as predator stimuli has been found to be an effective tool for examining antipredator behaviour (Rowland, 1999; Corkum, 2002). The model was suspended in the bottom third of the water column with monofilament line from a pulley system (Figure 1). During trials, we used a lever attached to the pulley system to move the model at approximately 0.25 m/s, along the broad side of the tank.

## 2.6. Statistical analyses

We used general linear models to examine variation in prey behaviour, predator behaviour, and prey mortality. Across the three experiments, we consistently examined variation in the four prey behaviours (activity, vertical distribution, habitat use, and grouping) with factorial MANOVAs and ANOVAs that tested for species, treatment, species  $\times$  treatment effects, and a time blocking factor when appropriate. These analyzes were performed using prey group means that were averaged over trial duration (i.e., the mean of all observations, Rehage et al., 2009), since single scans are not statistically independent (Martin & Bateson, 2007). For the cue experiments, we calculated

the difference between post and pre-stimulus behaviours, and performed analyses on these differences. Since prey were only used once, behaviours were averaged to obtain group means, and the measured behaviours are not mutually exclusive, we consider all behaviours measured to be independent (Martin & Bateson, 2007).

For experiment 1, we also conducted ANOVAs to compare prey mortality (factorial: prey species and predator treatment effects) and predator behaviour (one-way: predator treatment). The number of predators active, at the top of the water column, and in structure were averaged for each trial and compared across treatments. To satisfy normality assumptions, we examined residuals in all models, and transformed variables ( $\sqrt{y}$ -transformations for counts and  $\arcsin(\sqrt{y})$ -transformations for proportions) that showed evidence of non-normality or heteroscedacity (Kery & Hatfield, 2003). LSD pair-wise comparisons were used in post-hoc tests, and significance at the 0.05 level is denoted with letters in bar graphs. All analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA).

### 3. Results

#### 3.1. *Experiment 1: prey responses to predator presence & predation rates*

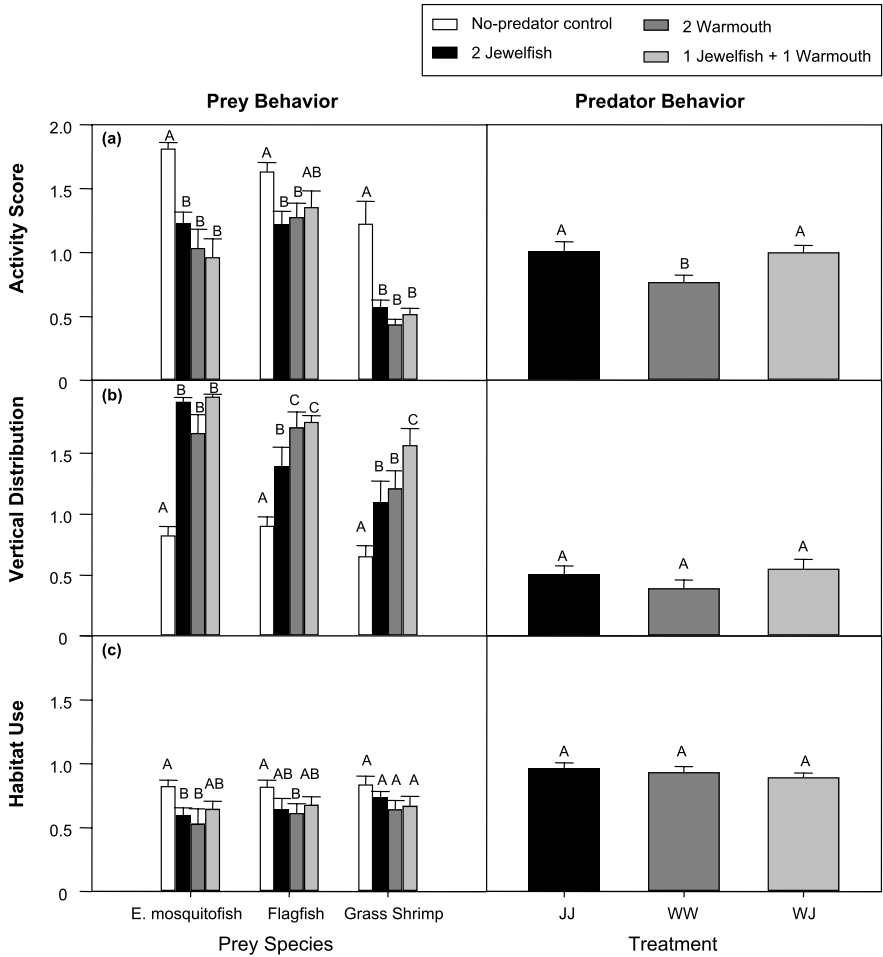
The three native prey species varied in activity and grouping behaviour, but showed similar microhabitat use. Overall, grass shrimp were less active and less likely to form groups than either mosquitofish or flagfish. Across predator treatments, the behavioural response of the three species was surprisingly similar (Table 1). For three of the four behaviours measured, we recorded consistent responses to the presence of predators, regardless of predator identity. All three prey species decreased activity, moved higher in the water column, and increased grouping in treatments in the presence of predators (Figures 2 and 3). Thus, contrary to expectations, prey responses to the native vs. the non-native predators were similar in strength and direction for all prey types.

The only exception was a differential response to predator treatments in the vertical distribution of prey (Figure 2). Mosquitofish moved higher in the water column regardless of predator treatment, but the response was dependent on predator identity for flagfish and grass shrimp. Shrimp showed a stronger response when predators were mixed, while flagfish showed

**Table 1.** Results of ANOVAs and MANOVAs ( $F$  values, degrees of freedom,  $p$  values and  $R^2$ ) testing treatment, species and block effects for the first predator–prey experiment.

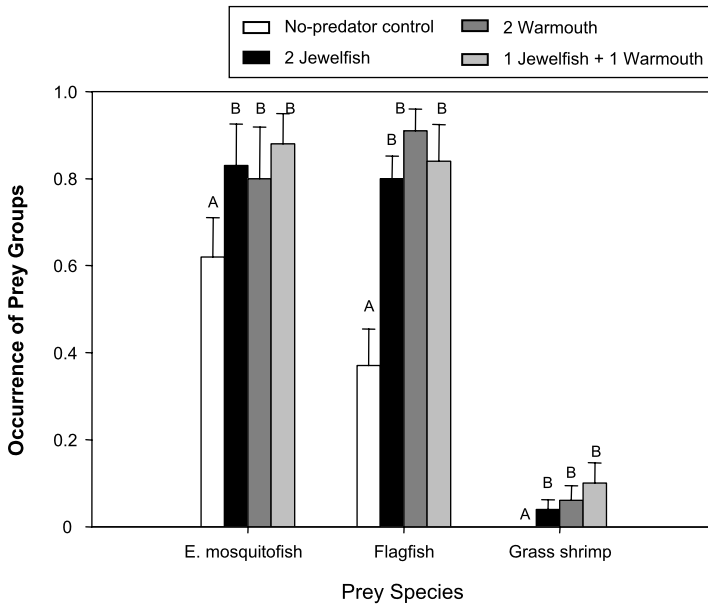
Predator–prey experiment	Predator treatment		Prey species		Predator treatment × prey species		Block		$R^2$
	$F$ (df)	$P$	$F$ (df)	$P$	$F$ (df)	$P$	$F$ (df)	$P$	
Prey mortality	157.4(3,96)	<0.001*	9.5(2,96)	<0.001*	3.3(6,96)	0.005*	0.3(1,96)	0.581	0.8
Predator behaviour									
Multivariate analysis	4.1(8,138)	<0.001*						0.5(4,69)	0.733
Activity	4.5(2,72)	0.015*						0.5(1,72)	0.504
Vertical distribution	1.2(2,72)	0.310						0.2(1,72)	0.684
Use of habitat									
structure	0.7(2,72)	0.490						0.3(1,72)	0.585
Predator–predator interactions	7.0(2,72)	0.002*						0.0(1,72)	0.878
Prey behaviour									
Multivariate analysis	14.4(12,246)	<0.001*	28.9(8,186)	<0.001*	1.6(24,326)	<0.044*	0.5(4,93)	0.766	
Activity	20.8(3,96)	<0.001*						0.1(1,96)	0.759
Vertical distribution	40.0(3,96)	<0.001*	50.5(2,96)	<0.001*	1.8(6,96)	0.102		1.1(1,96)	0.289
Use of habitat			17.1(2,96)	<0.0018	2.2(6,96)	0.005*			
structure	5.6(3,96)	0.001*	1.0(2,96)	0.372	0.2(6,96)	0.968		0.0(1,96)	0.958
Grouping	9.8(3,96)	<0.001*	131.2(2,96)	<0.001*	2.4(6,96)	0.034*		0.2(1,96)	0.657

\* Significant effect.



**Figure 2.** Mean predator and prey activity, vertical distribution, and structure use ( $\pm 1$  SE) for the first experiment across predator treatments (NP = no predators, JJ = 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Activity was scored 0–3 (0 = not active), vertical distribution was scored as 0–2 (0 = bottom), and structure use reflect counts of the number of prey individuals within the structure averaged over the observation period. Significant pair-wise differences ( $p \leq 0.05$ ) are indicated by different uppercase letters.

equally high responses with mixed or warmouth predators, but a lesser response when the predators were the jewelfish pair (Figure 2B). Little variation in use of the habitat structure was seen across treatments for shrimp, but a slight decrease was detected for the fish prey when predators were present

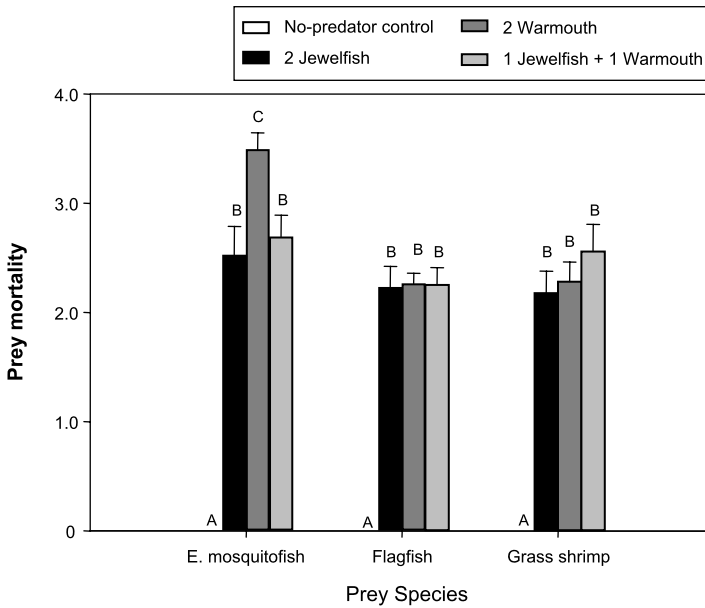


**Figure 3.** The mean occurrence of prey groups for the first experiment ( $\pm 1$  SE) across predator treatments (NP = no predators, JJ = 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Prey grouping was scored as 0 or 1 (0 = group absent, 1 = group present). Significant pair-wise differences ( $p \leq 0.05$ ) are indicated by different uppercase letters.

(Figure 2C). However, overall use of the structure was low; on average only one of the six individuals was found in the structure across treatments.

The predator pairs varied in activity, but showed similar patterns of microhabitat use in our experimental tanks (Table 1, Figure 2). Warmouth pairs were the least active, whereas average activity levels were similar for the jewelfish pair and the mixed predator treatment. Across pair types, predators remained low in the water column and, on average, one of the predators spent the trial duration in the more complex artificial vegetation.

Predation rates varied as a function of predator treatments, prey species, and the predator treatments by prey species interaction (Table 1). As may be expected, mortality was higher in predator treatments (zero in the absence of predators), but highest in the warmouth treatment; 38% of prey were consumed in warmouth treatment relative to 33% consumed with mixed predators, and 29% with jewelfish (Figure 4). Consumption rates of flagfish and grass shrimp did not differ significantly among the treatments, but mortality



**Figure 4.** Mean predation rate ( $\pm 1$  SE) on all prey across treatments (NP = no predators, JJ = 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Letters indicate significant pair-wise differences at  $p \leq 0.05$ .

of mosquitofish was higher in the presence of the native warmouth pair than in the other two predator treatments.

### 3.2. Experiment 2: prey responses to chemical cues

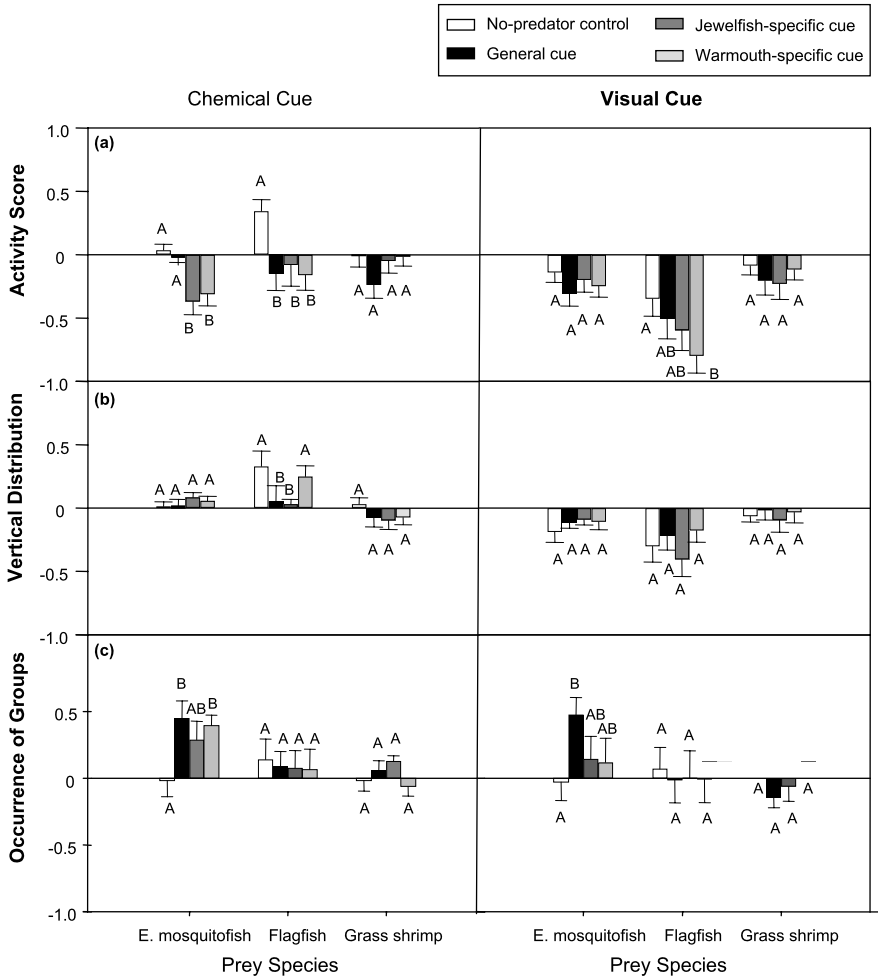
Overall, prey responses to chemical cues were relatively weak, showing prey-specific responses, and low differentiation among cue types (Table 2, Figure 5). For instance, grass shrimp did not respond to any of the chemical cues presented. Mosquitofish shifted activity and grouping behaviour when chemical cues were present, but few to no differences were detected among cue types. Mosquitofish became less active with the scent of warmouth and jewelfish, and increased grouping indiscriminately to both the general and the two specific chemical cues (Figure 5A,C). Flagfish became less active in response to all cue types, including the scent of novel jewelfish (Figure 5A,B). They moved lower in the water column with the conspecific cue and jewelfish scent, but not the warmouth scent.



**Table 2.** Results of ANOVAs and MANOVAs (*F* values, degrees of freedom, *p* values and *R*<sup>2</sup>) testing treatment, species and block effects for the two cue experiments.

	Predator treatment		Prey species		Predator treatment × prey species		Block		<i>R</i> <sup>2</sup>
	<i>F</i> (df)	<i>p</i>	<i>F</i> (df)	<i>p</i>	<i>F</i> (df)	<i>p</i>	<i>F</i> (df)	<i>p</i>	
<b>Chemical cue experiment</b>									
(prey behaviour)									
Multivariate analysis	2.0(12,278)	0.023*	3.7(8,210)	<0.001*	2.0(24,367)	0.006*			
Activity	5.9(2,108)	0.009*	2.4(3,108)	0.095	2.5(6,108)	0.024*			0.3
Vertical distribution	2.0(2,108)	0.106	9.3(3,108)	0.002*	1.5(6,108)	0.200			0.2
Use of habitat structure	1.4(2,108)	0.253	0.1(3,108)	0.893	1.6(6,108)	0.143			0.1
Grouping	4.0(2,108)	0.449	2.1(3,108)	0.022*	1.4(6,108)	0.388			0.1
<b>Visual cue experiment</b>									
(prey behaviour)									
Multivariate analysis	1.4(15,254)	0.170	5.6(10,184)	<0.001*	1.0(30,370)	0.551	0.9(5,92)		0.460
Activity	1.2(2,96)	0.318	22.1(3,96)	<0.001*	0.7(6,96)	0.600	3.0(1,96)		0.086
Vertical distribution	0.9(2,96)	0.446	7.3(3,96)	0.001*	0.5(6,96)	0.834	2.1(1,96)		0.148
Use of habitat structure	1.5(2,96)	0.231	2.6(3,96)	0.800	1.6(6,96)	0.150	0.0(1,96)		0.935
Grouping	0.2(2,96)	0.866	2.8(3,96)	0.069	1.2(6,96)	0.340	0.4(1,96)		0.538

\* Significant effect.



**Figure 5.** Mean predator and prey activity, vertical distribution, and habitat use ( $\pm 1$  SE) in the two cue experiments across predator treatments (NP = no predators, JJ = 2 jewelfish, WW = 2 warmouth, WJ = 1 jewelfish + 1 warmouth). Activity was scored 0–3 (0 = not active), vertical distribution was scored as 0–2 (0 = bottom), and the occurrence of prey groups was scored as 0 or 1 (0 = group absent, 1 = group present). Significant pair-wise differences ( $p \leq 0.05$ ) are indicated by different uppercase letter.

### 3.3. Experiment 3: prey responses to visual cues

Overall, prey behaviour in response to visual cues only did not vary strongly among prey, or more importantly among cue types, with two exceptions (Fig-

ure 5). Mosquitofish increased grouping in the presence of the fish model, and flagfish decreased activity strongly when warmouth were present in the adjacent tank (Figure 5A,C). There were some behavioural differences between pre and post cue delivery, but these differences were generally consistent across treatments including in the control tank, where neither predator nor predator model was present. Activity was lower across all three prey in the post-cue observations, and prey tended to move lower in the water column.

#### **4. Discussion**

Non-native predator effects are expected to be higher than those of native predators due to the lack of experience of the prey with the new predator, its foraging tactics, and cues (Cox & Lima, 2006; Banks & Dickman, 2007; Sih et al., 2010). Our experimental results with African jewelfish and Everglades prey, however, do not support this notion. First and contrary to expectations, the non-native jewelfish did not have a greater predatory effect on the three focal prey species tested relative to the native centrarchid predator. Second, our prey showed antipredator responses to non-native jewelfish that were generally similar in magnitude and direction as those exhibited toward the native warmouth. Lastly, two of the three prey species tested appeared to be able to detect and respond to olfactory cues from novel African jewelfish, despite having not encountered these predators nor olfactory cues before in nature. These results suggest that although prey may be faced with new predators, if these predators are somewhat similar to existing predation threats (i.e., other fish predators or confamilial predators), prey may exhibit general antipredator behaviours that are known to increase survival (e.g., reduced activity; Skelly, 1994).

Because of the naiveté of prey, introduced predators may have greater consumptive effects relative to non-consumptive effects when compared to native predators (Sih et al., 2010). These greater consumptive effects may explain the boom and bust cycles often associated with invasions (e.g., Bohn et al., 2008). In our trials, however, non-native jewelfish had similar or lower consumptive effects to those of a similar-sized native centrarchid. Foraging rates were similar on the two demersal prey, grass shrimp and flagfish, but varied for the top-dwelling mosquitofish. Jewelfish consumed less

mosquitofish, despite the fact that mosquitofish are a major component of jewelfish diets (W. Loftus, unpubl. data), and jewelfish consume them readily in the lab (Rehage et al., 2009). This is surprising given that both predators had similar microhabitat use in the lower water column, and would typically be expected to forage more effectively on prey that share the same habitat domain (Schmitz, 2007).

The shared prey and similarity in habitat use between the native warmouth and the non-native jewelfish supports the notion that native centrarchids, which are common mesoconsumers throughout Everglades habitats (Chick et al., 2004; Rehage & Trexler, 2006) are likely to compete for resources with non-native jewelfish (Schofield et al., 2007), as they do with other non-native cichlids previously introduced (Brooks & Jordan, 2010). However, we did not see any evidence of interference that would lead to risk enhancement or risk reduction when both predators were present (Sih et al., 1998; Schmitz, 2007). Predation rates in the mixed predator treatments were similar to those in single predator treatment, except for the lower predation rate on mosquitofish when predators were mixed.

Prey responded to the presence of predators with typical generalized antipredator behaviour (i.e., decreases in activity and increases in grouping, Sih et al., 2010), and these responses were similar to the native and non-native predators, and similar among the two fishes and shrimp prey. All prey became less active, moved higher in the water column, and increased aggregation in the presence of predators. Decreases in activity lead to a lower probability of detection by predators (Skelly, 1994), while schooling is known to enhance vigilance, and predator confusion, as well as dilute risk (Pitcher & Parrish, 1993). Movement up the water column has been previously reported in mosquitofish and other *Palaemonetes* spp. in response to fish predators (Main, 1987; Davis et al., 2003; Rehage et al., 2009); presumably as a habitat shift away from more demersal predators.

Due to their different morphologies and habitat domains, we expected to see more variation in prey antipredator behaviour. Even congeneric species of similar morphology and ecology show markedly different behavioural responses (Nannini & Belk, 2006). Antipredator responses typically relate to a species' history of exposure to predation risk and should influence their vulnerability to predators. Our results suggest that these species may experience similar predation risk in the field, and may be equally vulnerable to non-native predation threats.

Alternatively, it may be possible that the similarity in the behavioural responses observed in our trials are due to constraints provided by the experimental setup, which caused the prey to exhibit heightened and common generalized responses to a 'pulse' in predation risk (Lima & Bednekoff, 1999; Relyea, 2003; Schmitz, 2007). The effectiveness of antipredator behaviour is dependent not only on the identity of the predator and its foraging tactics, but also on the type of habitat where the predator is encountered (Brown & Smith, 1997). It is plausible that in the constrained space of lab aquaria, prey used generalized and stronger antipredator tactics to evade heightened predation risks since predator avoidance is limited (Hickman et al., 2004). Shifts in habitat use to predator-free environments will be limited under these lab conditions (Crowl & Covich, 1994). However, we believe our experimental setup had elements of reality. Most tank predation studies restrict predator movements, which generates limited behavioural responses, and prevent us from examining how predators and prey interact in space (Lima, 2002; Sih, 2005). By employing a free-ranging predator design, we were able to observe predator-prey encounters at close proximity, and quantify the behavioural response of prey given an encounter, but as in other studies, sacrificed the ability of prey to exhibit other spatial responses.

Yet, all else being equal, we expected to see differential behaviour toward the native and the non-native predator. We suggest three possible mechanisms for the similarity in response seen across the three prey types. First, we suggest that an adaptive evolutionary history with multiple predators may have allowed the prey to develop non-plastic behavioural traits in response to any predator threats (i.e., Sih, 1986). In general, fixed antipredator behavioural responses are expected to occur when predation risks are continuously high (Wolfahrt et al., 2006). In the Everglades, recurrent seasonal dry-down forces prey to live or move into deeper habitats where larger-bodied fishes are abundant and predation regimes are expected to be relatively high (Loftus & Eklund, 1994; Rehage & Trexler, 2006; Rehage & Loftus, 2007). This co-occurrence with predators may allow prey to exhibit similar antipredator responses to multiple threats, including those they have not encountered before. Sih (1986) found that predator-experienced prey had a greater chance of survival with novel predators than predator naïve prey, due to their fixed behavioural responses.

Second, prey species could be exhibiting a neophobic response, whereby they are responding to all things novel with aversion, hesitation, and/or caution (Greenberg, 2003). These responses are expected to be adaptive in high

predation risk environments, where larger fish, although not recognized, are likely to be a predator and elicit a response (Brown & Chivers, 2005). Thirdly, despite the fact that the prey used in our experiments were 'naïve' to jewelfish, since they had not previously encountered them in nature, jewelfish may not have represented a novel nor unfamiliar threat. In other words, jewelfish did not pose a unique or heightened threat for prey; thus, prey responded in a similar manner and with a similar magnitude as they would to familiar predators.

Prey exhibited antipredator behaviour in response to both general and specific cues, but mostly when these cues were chemical. A number of studies have documented the use of chemical cues in predator recognition (Mathis et al., 1993a; Mathis & Vincent, 2000), including those produced by non-native taxa (Pearl et al., 2003). Chemical cues likely provide an early warning of predation threats, which may be refined with the introduction of visual cues (Kats & Dill, 1998; Chivers et al., 2001). The low response to the visual cues used in our experiment, may be due to the fact that the visual cues used did not provide enough information for prey to correctly identify the predator threat (Wisenden et al., 2004), or they did not reflect a high risk encounter to merit a response (Corkum, 2002).

Prey often show a greater reliance on chemical cues when visual cues are diminished, such as in turbid waters, in heavily-vegetated habitats, or with cryptic predators (Hartman & Abrahams, 2000; Mathis & Vincent, 2000; Amo et al., 2004). Because of the high density of emergent grasses (Gundersen & Loftus, 1993), the high biomass of periphyton (Turner et al., 1999), and the presence of flocculent material atop the benthos (Rehage & Trexler, 2006), the structural complexity of Everglades marsh habitats is relatively high. Under these conditions, we expect prey to rely more intensely on chemical information as seen in our study (Mathis & Vincent, 2000). Similarly, several of the common native predators use a sit and wait hunting strategy, for which, it is more advantageous for prey to use chemical cues in predator recognition (Amo et al., 2004).

Prey may also be able to recognize not previously-encountered predators as a threat, if they are closely related to known predators (i.e., confamilial predators; Ferrari et al., 2007). Both mosquitofish and flagfish showed a decrease in activity and increase in vertical distribution to the isolated scents of jewelfish and warmouth. Often, the strength of a prey species' antipredator

response will depend on dietary cues, and whether the predator has consumed conspecific or heterospecific prey (Wohlfahrt et al., 2006), but we removed these cues from our experiment. Instead, we suggest that the fish prey may be relying on chemical kairomones for predator recognition and response. Kairomones are prominent chemical cues that are similar across freshwater fish families, and are believed to be a partial metabolite of fish-associated bacteria (Dicke & Sabelis, 1988; Elert & Phonert, 2000). Previous work shows that prey use these cues in predator recognition (Gelowitz et al., 1993; Kats & Dill, 1998). Kusch et al. (2004) showed that fathead minnow populations exhibited intense behavioural responses to increasing concentrations of northern pike (*Esox lucius*) odour and were able to recognize the size of the predators that generated the cues. The recognition of predator kairomones by prey can occur very quickly under natural conditions (Wisenden & Chivers, 2006). It may be possible that the prey's prior experience with other non-native cichlid predators may have allowed the fish prey to respond to non-native jewelfish. For instance, non-native Mayan cichlids (*Cichlasoma urophthalmus*) are a widely-distributed and longer-established (since 1983) generalist predator known to consume the focal prey species (Whitaker, unpubl. data). Ferrari et al. (2007) showed that fathead minnows trained to recognize the scent of a particular salmonid predator, also exhibited antipredator responses to the scent of two other salmonid species, despite no experience with them.

While chemical cues appear to be a primary source of information in predator-prey interactions in our trials, the antipredator responses observed during the cue experiments were weaker than those observed in the first experiment where predators were present. This supports the notion that prey need multiple cues to identify predation risk, and determine the degree of risk-sensitive behaviour to exhibit (i.e., threat sensitivity hypothesis, Amo et al., 2004; Botham et al., 2008). For instance, the relatively weak response of shrimp observed in the chemical cue trials may be due to the fact that they require other cue types, such as tactile cues. Crowl & Covich (1994) found that chemical cues elicit a partial response from freshwater shrimp, but when coupled with the physical presence of the predator the intensity of the responses increases. Mosquitofish similarly increased avoidance behaviour when both the chemical and visual cues of predatory fish were present (Smith & Belk, 2001).

Introduced predators are a major concern for the Everglades, and have been implicated in native population declines elsewhere in freshwater systems (Cox & Lima, 2006). With the continued invasion of new species, the probability for synergistic effects among fish predators that could drastically alter the way non-native species interact with natives and, thus, their impact (e.g., O'Dowd et al., 2003) becomes a concern. Our data show that a newly-arrived predator may have similar predatory effects and elicit similar antipredator behaviour from native prey as seen with a native predator. Thus, the vulnerability of Everglades prey to new predators does not seem to vary among taxa, and may be less than expected based on the novelty of the interaction, perhaps because of the prior experience of Everglades prey with cichlid predators. If predation rates and prey risk to non-native cichlids are similar, we would expect non-native predators to function in a similar matter as native predators. However, we do not know if the addition of non-native cichlids to the system is increasing overall predation regimes, with important consequences for the transfer of energy throughout food webs and ecosystem components, or replacing them. Further work is needed to distinguish between the two, and better assess the consequences of multiple invasions in the long-term.

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